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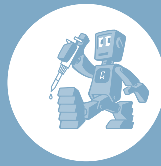

roboklon

dART RT Kit

– for Reverse Transcription Reactions –

dART RT Kit
for Reverse Transcription
and cDNA First Strand Synthesis

Cat. No.	Size
E0801-01	25 reactions
E0801-02	100 reactions

Storage Conditions:

Store at -20°C

Unit Definition:

One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble form in 10 min at 37°C (4).

Component	25 Rxn Kit	100 Rxn Kit
dART Reverse Transcriptase	25 µl	100 µl
5 x cDNA synthesis buffer	150 µl	600 µl
100 mM DTT	50 µl	200 µl
5 mM dNTPs mix	100 µl	4 x 100 µl
RNase Inhibitor (12.5 U/µl)	25 µl	100 µl
Oligo(dT)$_{20}$ (50 µM)	25 µl	100 µl
Random hexa-mers (50 ng/µl)	25 µl	100 µl
RNase free water	1.0 ml	4 x 1.0 ml
E.coli RNase H (2 U/µl)	25 µl	100 µl

Two-Step RT-PCR: Protocol Overview:

First step (this kit): cDNA synthesis starts from either total RNA or from poly(A)$^+$-RNA. Primers are oligo(dT), random hexamers or reverse (anti-sense) gene specific primers.

Second step: Aliquots of the generated cDNA serve as template for PCR reactions in separate reaction tubes. Specific primer pairs are used for dsDNA amplification. For molecular cloning we recommend using either PfuPlus (Cat. No. E1118) or Hybrid DNA Polymerase (Cat. No. E2950). For quantitative PCR methods, where PCR product generation is continuously monitored, the HotStart Perpetual Taq DNA (Cat. No. E2700) Polymerase is the polymerase of choice. For subsequent sequencing of amplified cDNA, we recommend using OptiTaq DNA polymerase (Cat. No. E2600).

Quality Control:

All preparations are assayed for contaminating endonuclease and exonuclease and nonspecific RNase and single- and double-stranded DNase activities.

The dART kit is optimized for high sensitivity RT reactions. It enables full length cDNA synthesis with high yield, even from rare, low copy number or delicate RNA templates. Featuring a carefully optimized, modified Reverse Transcriptase, the dART kit performs highly specific RT reactions.

Description:

→ For cDNA synthesis and for two-step RT-PCR reactions requiring high sensitivity and high specificity.

→ Greatly enhanced performance as compared to one-step RT-PCR reactions. No loss in RT sensitivity and no lowering of cDNA yield. Avoids any undesired compromises in reaction conditions, since RT and PCR reactions generally ask for very different buffer requirements.

→ Synthesizes single-stranded DNA from RNA templates in a broad range of temperatures between 35°C and 55°C.

→ High cDNA yield and full-length reverse transcripts for RNA templates with a wide range of G+C content. For sequence stretches with extremely high G+C content and very stable secondary structures we recommend using the thermostable AMV Reverse Transcriptase Native (Cat.No. E1372) at elevated reaction temperatures (55-65°C).

→ No detectable RNase activity for single-stranded RNA. Reduced RNase H activity (DNA/RNA hybrid molecules only). Since RT reactions are non-amplifying reactions, RNase H activity generally has no influence on cDNA product length and product yield.

→ Suitable for preparation of labeled hybridization probes.

dART Reaction Protocol:

This reaction allows synthesis of cDNA starting from small to medium RNA amounts. Depending on the abundance of the target RNA template in the RNA sample, from 10 ng up to 5 µg RNA permit the synthesis of full length cDNA.

1. Prepare RNA-Mix: For each reaction, combine these components in a 0.2-0.5-ml tube: 1 µl primer (50 µM Oligo(dT)$_{20}$ or 50 ng/µl random hexamer primer or 10 µM reverse gene specific primer), RNA, dNTPs mix. Adjust volume to 13 µl with RNase free water as follows:

RNA-Mix
Primer...1 µl
RNA (10 ng-5 µg)...x µl
5 mM dNTPs mix..4 µl
RNase free water...@ 13 µl
Total volume: 13 µl.

2. Optional step: Heat RNA-Mix for 5 min to 65°C and chill on ice for another 5 min *.

3. Vortex the 5 x cDNA synthesis buffer.

4. Prepare a **Master Reaction Mix** on ice and mix gently by pipetting on ice.

Master Reaction Mix
5 x cDNA synthesis buffer.................................4 µl
RNase inhibitor 12.5 U/µl..................................1 µl
100 mM DTT...1 µl
dART Reverse Transcriptase.............................1 µl
Total volume per reaction: 7 µl

5. Transfer 7 µl of Master Reaction Mix to each of the tubes containing 13 µl RNA-Mix. Total reaction volume: 20 µl. Keep on ice.

6. Transfer the sample from ice quickly into a preheated to appropriate temperature thermal cycler. Incubate as follows:

Oligo(dT)$_{20}$ primed	30-60 min at 50°C (or 35-55°C)
Gene specific primed	30-60 min at 50°C (or 35-55°C)
Random Hexamer primed	25°C for 10 min, followed by 20-50 min at 50°C (or 35-55°C).

7. Terminate the reaction by incubating at 85°C for 5 min.

8. Add 1 µl of RNase H and incubate at 37°C for 20 min (optional)..

9. cDNA is ready for PCR, can be used immediately or stored at -20°C.

10. Use 2 - 5 µl of cDNA as template for PCR reactions.

* The heating step is optional. Applied in case of difficult RNA templates or strong secondary structures, this step can improve results greatly. For all other templates, the heating step does not change reaction efficiency and can be omitted.

ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | D-13125 BERLIN | GERMANY
FAX 030 / 310 19 19 7 | PHONE 030 / 318 09 37 2 | WWW.ROBOKLON.DE
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